Technical

The Hydrocarbons of Anhydrous Butterfat: Influence of Technological Treatments

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ABSTRACT

The hydrocarbons of butter oil were isolated after saponification of the oil by thin layer chromatography and then analyzed by gas liquid chromatography. The influence of such treatments of butter oil as fractionation, passage over Fuller's earth, and hydrogenation on the nature and relative proportions of of the hydrocarbons was studied. Passage of butter oil over Fuller's earth causes the formation of cholesta-3,5-diene, probably from cholesterol. Hydrogenation of butter oil passed over Fuller's earth progessively transforms cholestadiene into cholestene.

INTRODUCTION

Recent work (1-5) has furnished precise data on the composition of the natural hydrocarbons of butterfat. Hydrocarbons constitute ca 0.1% wt of the butterfat, i.e., ca. 25% of the total unsaponifiable fat. Squalene is the predominant hydrocarbon. It constitutes 25 to 30% of the total hydrocarbons. In addition, a group of three recently identified hydrocarbons (1,5,6) constitute a relatively large proportion of the total hydrocarbons. These three hydrocarbons are (in the order of elution down a polar column, Carbowax 20 M or butanediol succinate) phytene-1 (3,7,-11,15-tetramethyl-n-hexadec-1-ene), phytene-2 (3,7,11,15-tetramethyl-n-hexadec-2-ene) and neo-phytadiene (3-methylene-7,11,,15-trimethylhexadec-1-ene).

Saturated aliphatic hydrocarbons comprise hydrocarbons with 14 to 35 carbon atoms, but hydrocarbons with 33, 34 and 35 carbon atoms are present only in trace amounts. Hydrocarbons with an uneven number of carbon atoms predominate (for example 25, 27 and 29 carbon atoms). Monoene and branched hydrocarbons are present in small quantities.

The relative proportions of hydrocarbons vary with the season. Phytene-1 and neophytadiene, or group "olefine II" of Ristow and Werner (3) are found in large quantities during the period at pasture (5) while, for example, hydrocarbons with 29 and 31 carbon atoms are in high quantities during the winter period (3,4). The proportion of squalene is relatively constant.

It is current practice to prepare anhydrous butterfat or butter oil from cream or butter, and it has become usual to submit this anhydrous butterfat to processing which modifies its physical and/or chemical properties. The purpose of this new technology is to increase the possibilities for utilization of surplus butterfat.

The present work is concerned with a study of the influence of selected treatments on the composition of hydrocarbons of anhydrous butterfat. The treatments applied in the present study have been fractionation of anhydrous butterfat after crystallization, hydrogenation and refining.

MATERIALS AND METHODS

Samples

Analysis was made on samples of anhydrous butter-fat of varying origins and different kinds, as follows: normal butter oils of several sources; butter oils of high melting points obtained by fractionation after crystallization; mixtures of normal and hydrogenated butter oil; butter oil decolorized then progressively hydrogenated. A comparative analysis was made, in addition, of a sample of natural tallow (beef suet), obtained by rendering of fatty tissues sampled at slaughter, and a sample of industrial refined tallow.

Isolation of Hydrocarbons

First method. After saponification of 15 g of fat, the unsaponifiable fraction is extracted using three times 50 ml of pentane washed with an aqueous solution of alcohol (50:50). The recovered unsaponifiable fraction, concentrated to 1 ml, is placed on a column (diameter 1.0 cm) containing 10 g of aluminum oxide deactivated by the incorporation of 3.5% water. Forty ml of pentane is used for elution. The eluate is concentrated to 1 ml.

Second method. Saponification is carried out according to Schwartz et al. (7). The unsaponifiable fraction is then fractionated by thin layer chromatography using Silica Gel G (developing solvent, for example, benzene/methanol 100:1.8). After scraping off the gel and elution using ethyl ether, the solvent is evaporated and the hydrocarbons recovered.

Analysis by Gas Liquid Chromatography

This was carried out in two different ways.

Conditions 1. A column 2 m long and about 2 mm internal diameter (1/8") filled with chromosorb W impregnated with 3% SE30; analysis at 210 C or 230 C under isothermal conditions or with a programmed temperature rise from 130 C to 245 C (at 4 C per min, for example).

Conditions 2. A column 2 m long and about 2 mm internal diameter (1/8") filled with chromosorb Q (80 to 100 mesh) and impregnated with 3% JXR; analysis under isothermal conditions at 240 C or with a programmed temperature rise from 170 C to 270 C (increase of 5 C per minute, for example).

RESULTS

Hydrocarbons of Normal Butter Oil

Figure 1 shows a chromatogram corresponding to the analysis of the hydrocarbons of a normal butter oil, obtained under programmed temperature conditions, and of a very high sensitivity, on stationary phase of JXR. The minor hydrocarbons, unsaturated and branched, are very distinct.

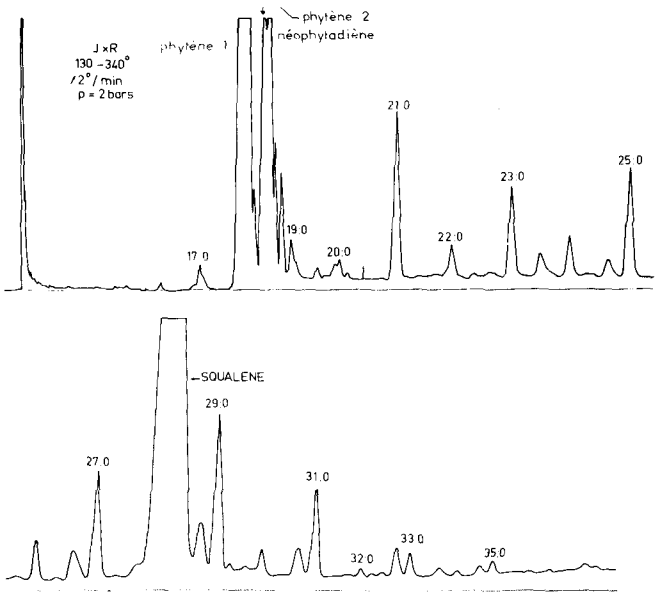

FIG. 1. Analysis by gas liquid chromatography of the natural hydrocarbons in the butterfat of a sample of cow's milk. Conditions of the analysis: programmed temperature rise from 130 C to 340 C (increase of 2° per min on 3% JXR).

Altogether 48 samples of butter oil have been analyzed. The hydrocarbon pattern is always the same. However, there are minor variations in the relative proportions of saturated hydrocarbons with 25 to 31 carbon atoms.

Influence of Fractionation by Crystallization of Butter Oil

The hydrocarbons of butter oil of high melting point (HMP) are hardly different from normal butter oil. The only difference is slightly smaller proportion of squalene.

Influence of Hydrogenation of Butter Oil

Hydrogenation modifies the nature and relative proportions of the hydrocarbons of butter fat. This is evidenced by the appearance of several peaks which precede the squalene peak. One occurs between the peaks of hydrocarbons with 25 and 27 carbon atoms.

The addition of only 1% hydrogenated butter oil to normal butter oil causes perceptible modifications. This is shown in Fig. 2.

The peak which appears on hydrogenation of butter and which occurs between the peaks corresponding to the hydrocarbons of 25 and 27 carbon atoms has not been proved to be caused by squalane. In fact, analysis by gas liquid chromatography of the hydrocarbons of a partially hydrogenated butter oil, to which squalane had been added, shows the persistent peak of the unknown hydrocarbon beside the peak of squalane.

Influence of Refining on the Composition of the Hydrocarbons of Butter Oil

Refining of an oil is carried out generally by passage over Fuller's (decolorizing) earth.

Figure 3 shows the superimposed chromatograms obtained from the hydrocarbons of a normal butter oil (chromatogram B) and from the hydrocarbons of this same oil decolorized by means of 1% decolorizing earth at 85 C for 15 min (chromatogram A).

The appearance of a hydrocarbon having retention time greater than that of squalene is the consequence of treatment of the oil by decolorizing earth. Some "butters" commercialized for puff pastry and analyzed previously also contained this component.

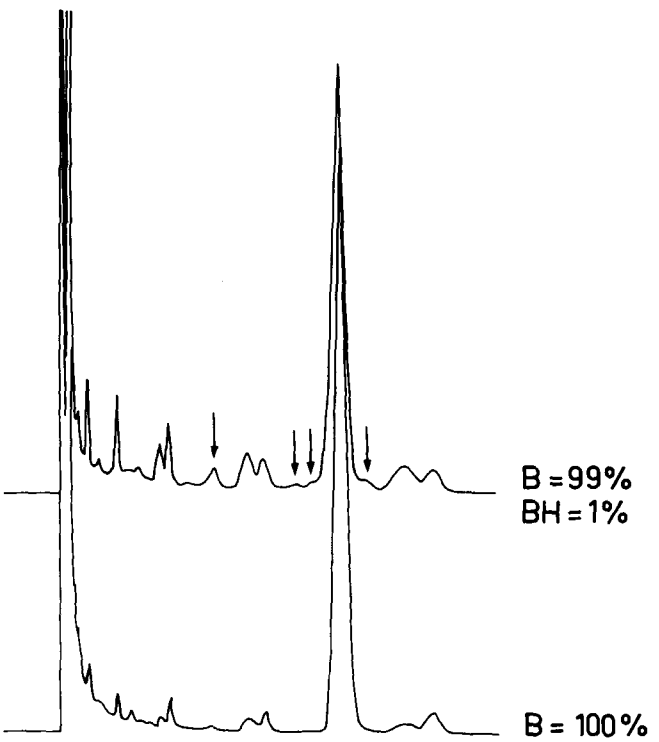

FIG. 2. Comparative analysis by gas liquid chromatography of the hydrocarbons of a normal butter oil (B = 100%) and of a normal butter oil to which 1% of hydrogenated butter oil had been added (B = 99%, HB = 1%). The arrows indicate the positions where new peaks appear (230 C – 3% SE30).

Finally, the comparative study of the hydrocarbons of a native beef tallow and an industrial refined tallow shows the presence only in the latter of a significant quantity of this unknown hydrocarbon.

The latter is easily isolated by thin layer chromatography of the total hydrocarbons (Silica Gel G and developing solvent, hexane). Its polarity is intermediate between the polarity of squalene and that of monoenoic aliphatic hydrocarbons. When submitted to analysis by ultraviolet spectrophotometry, this compound shows a very strong absorption at 232 nm, indicating the presence of conjugated double bonds.

The mass spectrum of this compound is given in Fig. 4 (lower part). Examination of this spectrum suggests that the compound has a steroid structure. In fact, the fragments of mass 255 and 213 are (as near as 2 units, i.e., as near as a double bond) the 257 and 215 peaks characteristic, on the one hand, of the four rings of a steroid containing, in addition the two methyl groups, i.e., $C_{19}H_{29}$ (in the present case $C_{19}H_{27}$), and, on he other hand, of the three rings of 6 carbon atoms, i.e., $C_{16}H_{23}$ (in the present case $C_{16}H_{21}$).

The position of the second double bond (the first being the double bond at 5 of the steroid nucleus) is not on carbon 2 of the steroid because, in this case, a compound corresponding to the elimination of butadiene, giving a peak at 314 (368-54) would be found during fragmentation.

The difference between the molecular mass 368 and the cyclic grouping 255 gives 113. This number corresonds to the mass of an 8 carbon saturated chain with a CH_3 terminal.

The rough formula of the hydrocarbon is therefore $C_{19}H_{27} + C_8H_{17} = C_{27}H_{44}$. It is a hydrocarbon with a steroid structure similar to cholestane ($C_{27}H_{48}$), but possessing two double bonds. One is on carbon 5, the other

is conjugated; if it is at 3, the compound is cholesta-3,5-diene.

The mass spectrum of cholesta-3,5-diene control is similar to the mass spectrum of the compound studied (8).

Thus, cholesta-3,5-diene is formed in quite large quantity during refining of an animal fat and, more exactly, during treatment with a decolorizing earth.

Influence of Hydrogenation after Refinement

A butter oil that had been subjected to a decoloration was then progressively hydrogenated under industrial hydrogenation conditions. Samples were taken every five minutes. The results of analysis of the hydrocarbons in the various samples by means of gas liquid chromatography (on stationary phase SE30) are given in Fig. 5. The formation of cholestadiene may be observed as soon as refining was first brought into operation, followed progressively during hydrogenation by the appearance of a more saturated compound of which the retention time is less than that of cholesta-3,5-diene.

Identified by mass spectrometry, this hydrocarbon, formed by hydrogenation of cholesta-3,5-diene, is cholestene (containing traces of cholestane). The mass spectrum of the cholestene formed appears in Fig. 4 (upper part).

DISCUSSION

Information on the hydrocarbons of butter or animal fats gives useful information that may lead to controlling their composition. Thus, modification of the natural hydrocarbons of butter by hydrogenation allows, for example, the differentiation of a butter of a high melting point obtained by fractionation from a butter of an equally high melting point obtained by partial hydrogenation.

The "creation" of a new hydrocarbon by a treatment such as passage over Fuller's earth allows butter oils which

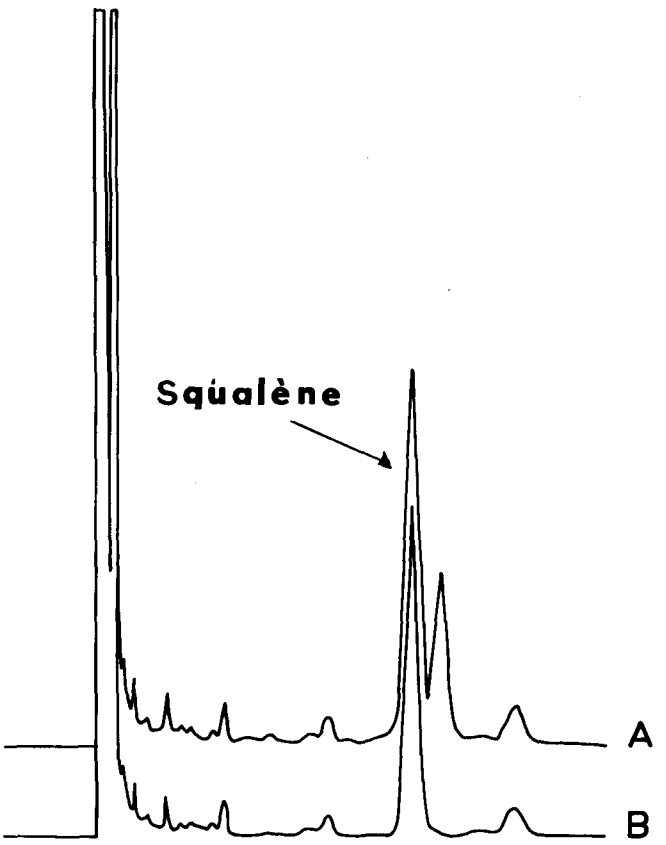

FIG. 3. Comparative analysis by gas liquid chromatography of the hydrocarbons of a normal butter oil (B) and a butter oil which had been subjected to treatment by passage over Fuller's earth (A). A peak may be observed following the squalene peak.

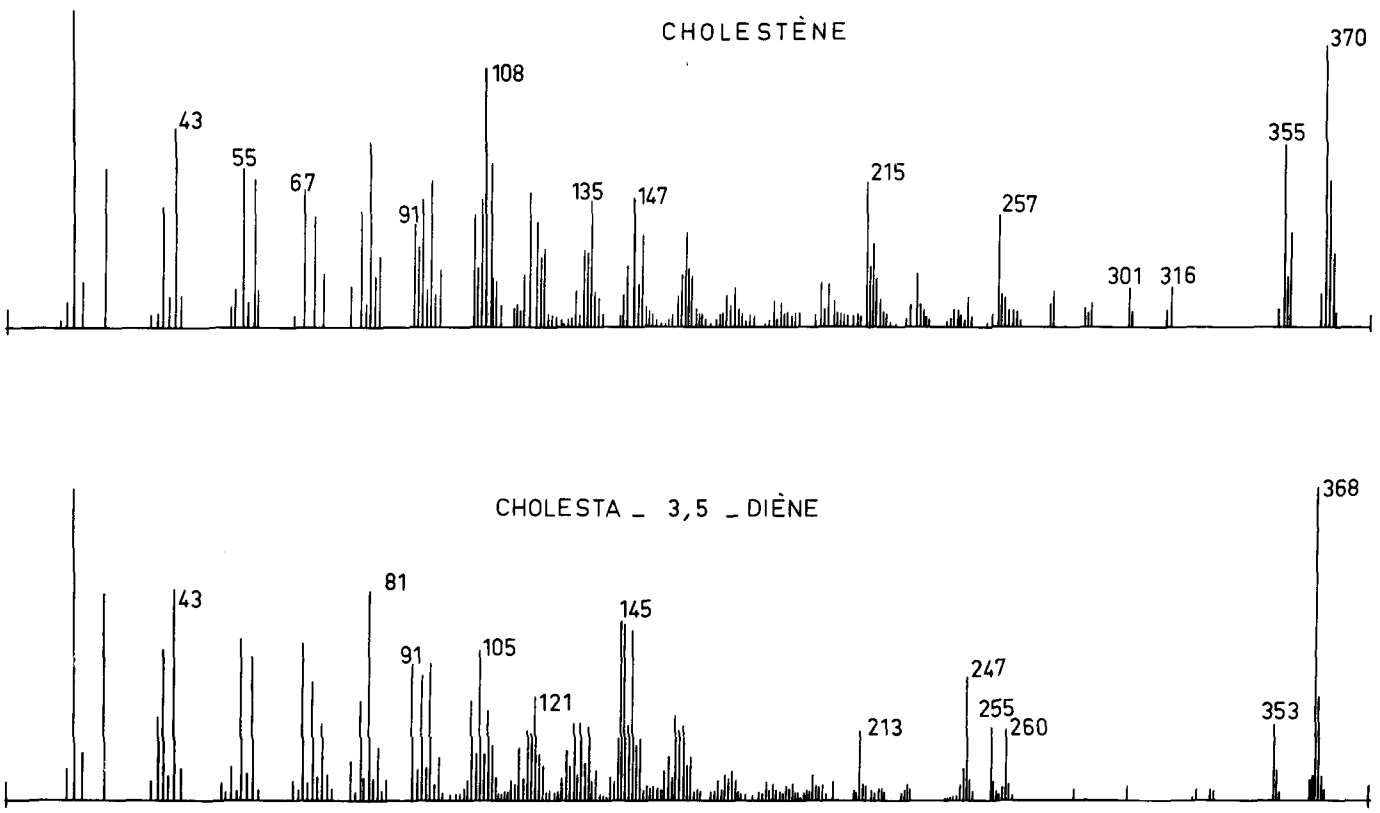

FIG. 4. Reproduction of mass spectra; of cholesta, 3-5, diene formed from cholesterol during passage over Fuller's earth (lower part); of cholestene formed from cholestadiene during hydrogenation of butter oil passed over Fuller's earth (upper part).

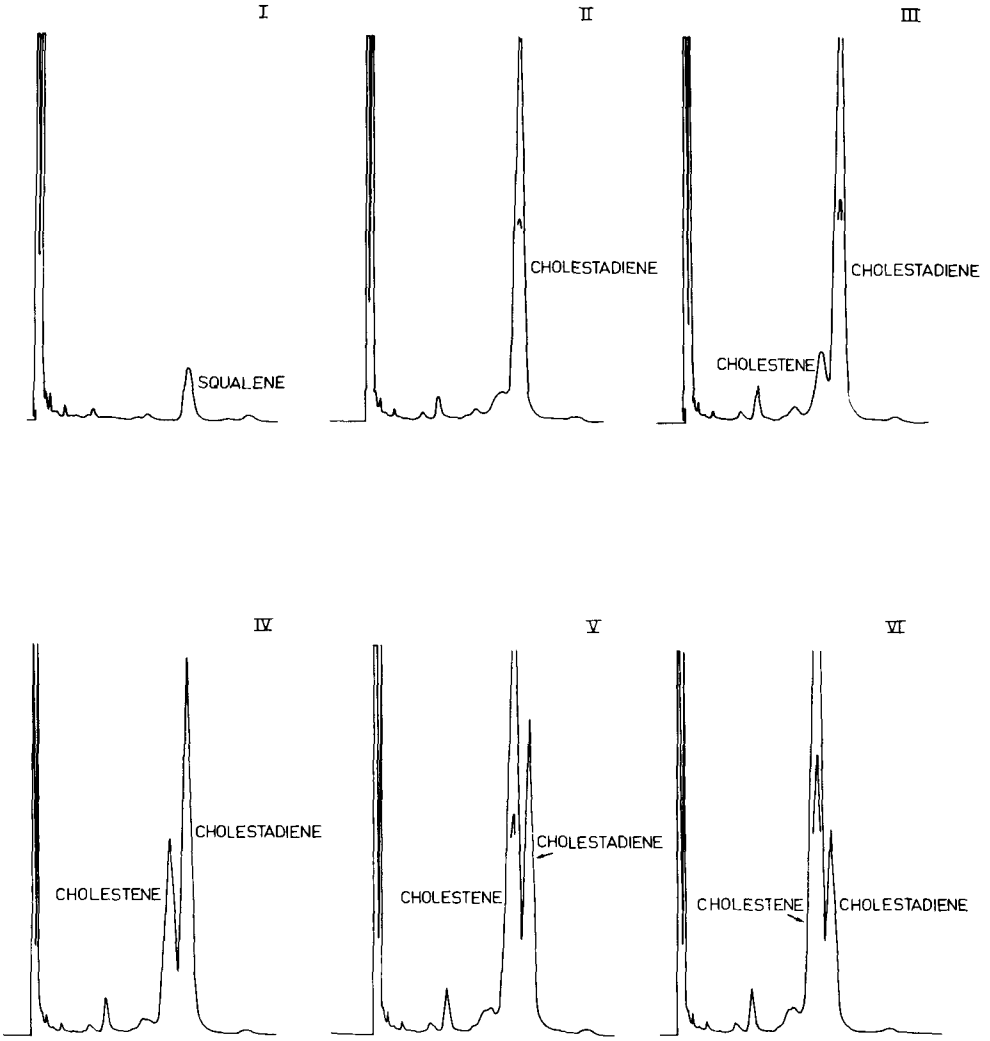

FIG. 5. Progressive transformation of the hydrocarbons of a butter oil: I hydrocarbons of butterfat before any treatment-iodine value: 36.7; II the same as I after bleaching on 2% of activated earth at 80 C for 15 min – iodine value 36.1; III, IV, V, and VI transformation of the hydrocarbons after 5, 10, 15 and 20 min of hydrogenation to an iodine value of 34.0 – 31.9 – 28.0 – 25.4 respectively. Condition of hydrogenation: 180 C – 0.3% nickel catalyst.

have been subjected to such a treatment for one reason or another to be recognized. Butter, sold as it is, obviously should not have been subjected to any process of refining. The fact that butter is not refined is a characteristic in its favor.

Moreover, it would be interesting to evaluate precisely the decrease in the quantity of cholesterol in butter oil or in tallow subjected to a decolorization treatment.

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